Spring 2015

Formation of Polymer Micro-Tubes

Daniel Peters
dgp7@zips.uakron.edu

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Daniel Peters

Department of Chemical and Biomolecular Engineering

Honors Research Project

Submitted to

The Honors College

Approved:

Bi-min Zhang Newby Date 4/13/15
Honors Project Sponsor (signed)

Bi-min Zhang Newby
Honors Project Sponsor (printed)

Date 4/13/2015
Reader (signed)

Gang Cheng
Reader (printed)

Date 4/13/2015
Reader (signed)

Edward A. Evans
Reader (printed)

Accepted:

Date 13 APR '15
Department Head (signed)

H. MICHAEL CHEUNG
Department Head (printed)

Date 4/13/15
Honors Faculty Advisor (signed)

Bi-min Zhang Newby
Honors Faculty Advisor (printed)

Date
Dean, Honors College

Abstract

Current demand for organs is greater than the supply of donated organs, which means that a new method of obtaining replacement organs needs to be found. The objective of the research is to evaluate the use of thermo-triggered self-rolling polymer tubes that can be used for scaffolds in tissue engineering. Additionally, the research also includes a method for forming polymer tubes through the removal of a sugar fiber, coated with a thin film of polymer, by dissolving it in deionized water. For the first approach, the effect of the aqueous environment and temperature on a polymer bilayer was observed in this research. In particular, the effect of the aqueous environment on the self-rolling ability of the polymer bilayer. Also, the effect of temperature on the swelling of the active gelatin layer. The active gelatin layer had a thickness of 577 nanometers and the passive polystyrene layer had a thickness of 275 nanometers. It was observed that with increased temperature the tube would begin to roll from end to end but, would not form a complete tube. For the second approach, hollow polymer tubes with diameters on a micrometer scale were easily produced. The self-rolling polymer micro-tubes were unsuccessful, simpler methods of polymer tube formation should be further sought to encapsulating cells for tissue engineering.

Executive Summary

The challenge of regenerative medicine in the modern era is the reformation of natural tissue because of the complexity involved with tissue regeneration such as microstructure and multicellular composition. In order to design a scaffold that can provide a suitable environment for cell deposition the scaffold must be biodegradable, biocompatible, and porous in order to allow the cells to assimilate to the body's natural environment. The purpose of this report was to investigate the encapsulation of cells using self-folding polymer micro-tubes. These polymer micro-tubes may be used as scaffolds for three-dimensional tissue constructs, which is a better alternative to the traditional two-dimensional cell culture approach. Another purpose of this study was to investigate a simpler method of polymer tube formation through the removal of a sugar fiber, with a polymer thin film coated on it, by submersing it in an aqueous environment.

This research found that the polymer bilayer consisting of an active gelatin layer (thickness = 577 nm.) and a passive polystyrene layer (thickness = 275 nm.) does not form self-folding polymer tubes under aqueous conditions. It was observed that with increases in temperature, the polymer bilayer would begin to roll from end to end, but was not able to form the tube. When the aqueous environment reached a temperature near body temperature, the gelatin layer dissolved and the polystyrene layer returned to the initial undeformed state.

This research also found that the method of drawing out a sugar fiber with a needle tip and coating this fiber with polymer was a valid approach to forming a polymer tube. In order

to create the polymer tube, the initial sugar fiber is dissolved in deionized water and a polymer tube with an average inner diameter of 232.9 micrometers can be found.

This project needed many new skills in order to deposit polymer solutions on substrates and characterize the final results of the experiments. One skill needed was spin-coating thin films on glass substrates by depositing a small amount of polymer solution on a glass slide and running a machine at 2000 rpm's for 30 seconds. Additionally, this study required analysis through ellipsometry, which is a non-destructive way of measuring the thickness of thin films on silicon wafers. Finally, this research required the use of optical microscopy in order to visually see the physiological changes taking place within a sample during the experiment.

In the future, more polymer bilayers should be evaluated in order to find a working method for producing stimulus-triggered self-folding polymer micro-tubes. Also, the encapsulation of cells should be studied because this possibility could lead to effective tissue engineering which may save lives. Furthermore, the insertion of cells into the polymer tubes formed by the removal of a sugar fiber should be studied in order to see if this method has validity in tissue engineering.

Introduction

Tissue engineering is the study of the growth of new connective tissues or organs from cells and a scaffold to produce a fully functional organ for implantation back into the donor host. One of the greatest challenges in tissue engineering is the formation of a scaffold that is biocompatible, biodegradable, porous, and the proper environment for mimicking the properties of the extracellular environment. One approach to the development of biocompatible scaffolds is the use of self-rolling polymer systems where the difference in mechanical properties of polymers can cause a rolling motion in a polymer bilayer. The self-rolling polymer tubes can be used as synthetic scaffolds for small diameter artificial blood vessels (6). These artificial blood vessels are paramount in wound healing as it would provide the wound with blood needed to form a clot, and could replace an existing artery that has experienced thrombosis, which could prevent a heart attack (7). The self-folding action can be induced by several stimuli's such as ultraviolet light, temperature, or pH. This method of self-rolling polymer bilayers can be used in the encapsulation of cells which offers a new advantage to industry as the fabrication step is separated from the encapsulation step. Additionally, another method for the formation of polymer tubes, is the drawing out of a sugar fiber then coating that fiber with a biocompatible polymer and finally dissolving the sugar fiber, leaving a polymer tube.

The purpose of this project was to form polymer tubes by coating a glass slide with an active polymer such as gelatin and then coating a polystyrene layer on top of the gelatin layer. The gelatin layer would act as the active layer which means it would undergo a physiological change in response to a stimulus. Additionally, this project looked for additional methods of polymer tube formation for the encapsulation of cells. One method that was used was the coating of a sugar fiber with a biocompatible polymer and then dissolving

the sugar fiber in water to leave a polymer tube. The formation of these polymer tubes is important because they may be used as scaffolds for cell encapsulation which would lead to the creation of artificial tissues or arteries.

Background

One concept studied by this project was microoragami or the formation of three-dimensional objects through the bending moment created by a polymer bilayer of thin films. Previously, this concept has been used to create polymer micro-tubes through the design of semiconductor and metal-oxide self-folded tubes for the creation of energy storage elements and a platform for investigation of behavior of confined cells (2-3). Another use of inorganic self-rolled polymer tubes has been studied for purposes such as microsurgery and controlled encapsulation of drugs which are then used for drug delivery (4-5). However, both of these studies used the application of inorganic self-rolling films which has limited use in the biotechnological field because of the poor biocompatibility and the rigidity of the materials used in this application.

Polymer based scaffolds are more suitable for biotechnological constructs because they have the ability to undergo reversible changes such as folding and unfolding in response to a certain stimulus such as temperature. Additionally, several biocompatible polymers are available and have already been approved for their use in medicine. Furthermore, since the polymers can be patterned using different techniques such as photolithography or physical cutting, a number of different two-dimensional shapes may be created in order to encapsulate cells that will be used in tissue engineering. The design of self-folding thin bilayers are generally based on strain-generated bending moments that transform the two-dimensional bilayers into three-dimensional objects (1).

The gelatin used as the active layer of the polymer bilayer came from bovine and porcine skin. Gelatin is a hydrolyzed form of collagen that is used for its biocompatibility and biodegradable properties. Additionally, polystyrene is a synthetic polymer made from the

monomer styrene and the most important property is the glass transition temperature which is stated to be 100°C (8). Furthermore, poly (l-lactide-co-glycolide) was used for its biocompatibility and low glass transition temperature of 40°C.

Another method for the formation of polymer tubes for cell encapsulation is through the coating of a polymer on a fiber and removal of fiber to form a hollow polymer tube. This method uses a fiber that is easily dissolved when introduced to a certain solution or stimulus.

Experimental Section:

Preparation of gelatin solution

Using a scale, 0.2571 grams of gelatin from bovine and porcine bones was measured out. Also, 2.2244 grams of water was weighed out, then the gelatin was added to the water in order to make a ten weight percent solution of gelatin. This solution was placed on a hot plate with a temperature of 40°C in order to create a homogeneous mixture.

Preparation of Polystyrene Solution

First, 0.132 grams of polystyrene was weighed out in a weighing dish, then 2.501 grams of toluene were weighed out in a volumetric cylinder. Next, the polystyrene was added to the toluene and placed on a magnetic stirrer in order to allow the polystyrene to dissolve and form a homogeneous solution.

Preparation of Glass Substrate

Glass slides were cut into rectangles using a diamond pen and submerged in a piranha solution that contained 30% hydrogen peroxide (H_2O_2) and 70% sulfuric acid (H_2SO_4) for 30 minutes. Subsequently, the glass slides were rinsed with deionized water and stored in deionized water for no longer than 1 week.

Polymer Films Deposition

The gelatin solution was deposited on the glass substrate using spin-coating which was set at 2000 rpm's for 30 seconds. Using ellipsometry, the gelatin film was found to have an average thickness of 577 nanometers. After spin-coating the gelatin layer was allowed to dry in air for 1 hour. The polystyrene solution was dip-coated onto the glass substrate through manual submersion of the substrate for 30 seconds. Also using ellipsometry, the polystyrene layer was found to have a thickness of 275 nanometers. The polystyrene layer

was allowed to dry under the hood for 1 hour in order to make sure the solvent was no longer on the glass substrate. The general scheme of polymer bilayer formation is shown in figure 1. First, the active or responsive polymer (gelatin) is deposited on the glass substrate. Then, the passive polymer (polystyrene) is deposited from a selective solvent (toluene) on top of the first polymer layer. The patterning of the polymer bilayer was achieved using physical cutting with a clean, new blade.

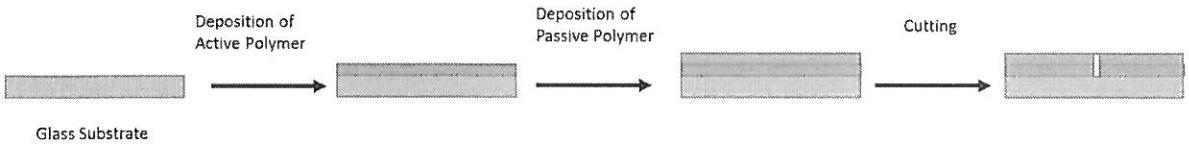

Figure 1: Scheme of patterned polymer bilayer film formation.

Formation of Polymer Tubes

Fabrication of the polymer tubes was attempted by exposure of the patterned bilayer to deionized water at temperatures ranging from room temperature (22°C) to about body temperature (37°C).

Preparation of Sugar Fiber

The pure cane sugar was placed on a hot plate with a temperature of about 200°C in order to melt the sugar. Then, a small needle was used to draw the sugar out into a thin fiber which was allowed to cool in air for one minute. Next, the sugar fiber was cut using a clean, new blade.

Preparation of Poly (Lactide-co-Glycolide) Solution

Using a 2.5 weight percent solution that was already mixed, pure poly (lactide-co-glycolide) was added in order to create a 5.0 weight percent solution in ethanol. The solution was placed on a magnetic stirrer in order to form a homogeneous solution.

Formation of Polymer Tube from Coated Sugar Fiber

The general scheme for polymer tube formation using the sugar fiber can be seen in figure 4. In order to coat the sugar fiber, the poly (lactide-co-glycolide) was dip-coated on top of the fiber by dipping the fiber in the solution for 30 seconds. The coated fiber was allowed to dry in air for 15 minutes to allow the solvent to evaporate from the surface of the fiber. Finally, the coated fiber was introduced to deionized water causing the sugar to evaporate and forming a hollow polymer tube.

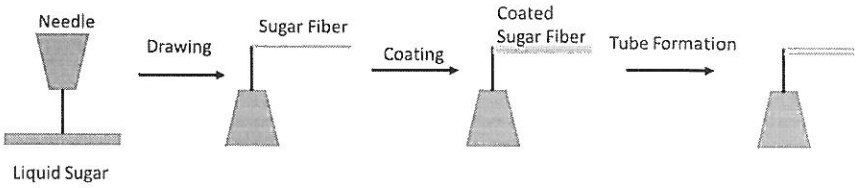

Figure 2: General scheme of polymer formation with sugar fiber.

Characterization Techniques

The thickness of dry polymer layers was determined using ellipsometry which is an optical technique that uses the change in the state of light polarization upon reflection from a sample to characterize the thickness of the thin polymer layers. In order to perform the ellipsometry, the polymer must be coated on a silicon wafer and this method is only valid for single polymer layers.

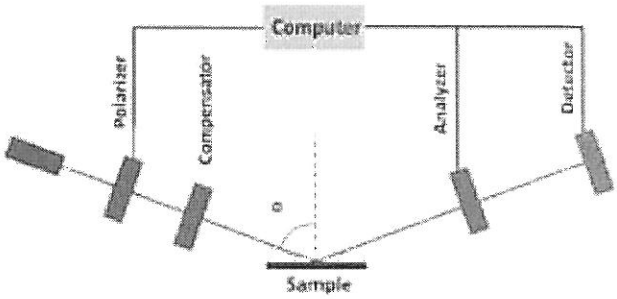

Figure 3: Simplified principle of ellipsometry (1).

The primary setup for ellipsometry includes a light source such as a laser, a polarizer, a sample, an analyzer, and a detector. In order to use the ellipsometry the optical constants of materials used need to be known in order to analyze the results because this is an indirect method of measuring thickness.

Another characterization method used in this study was the use of optical microscopy which is a method used to produce magnified photographic images of objects that are hard to see with the human eye. This method utilizes the principles of transmission, absorption, diffraction, and reflection of light waves. The optical microscope produces an image based on the magnification used and the ability of the microscope to focus the details of the object.

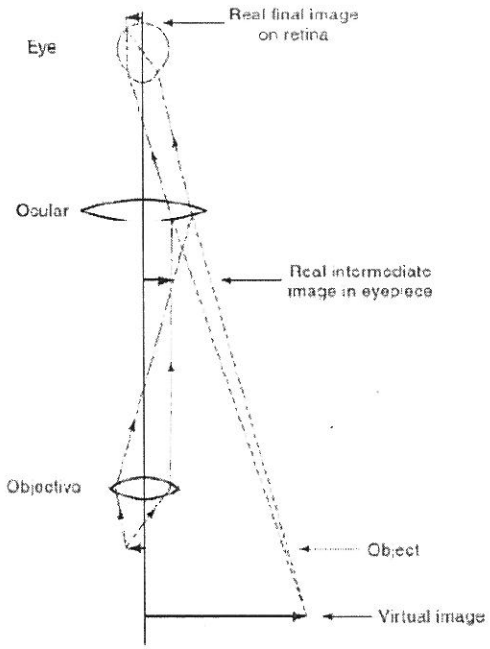

Figure 4: Perception of a magnified virtual image in the microscope (1).

The microscope's pathway consists of a light source, condenser, sample, objective, eyepiece, and detector such as a camera.

Results

Formation of Self-Rolled Polymer Tubes

This study investigated the formation of thermos-responsive self-rolled polymer tubes in an aqueous environment. The polymer bilayers were prepared on a glass substrate using thermo-responsive gelatin as the active layer and rigid polystyrene as the passive layer. The polymer bilayer remained undeformed while in a dry state. Once the polymer bilayer is introduced to an aqueous environment, the gelatin begins to swell while the passive polystyrene layer restricts the swelling of the active layer. As a result of this inhomogeneous swelling, the patterned bilayer will fold in order to form a self-rolled tube. In theory, as the temperature is increased to body temperature, the gelatin layer should disappear and the tube will unroll.

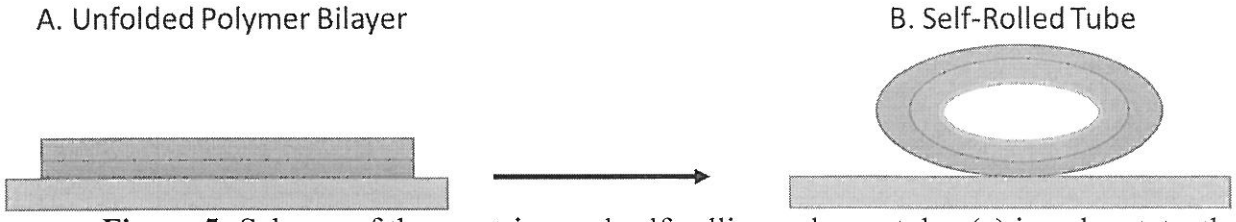

Figure 5: Scheme of thermo-triggered self-rolling polymer tube: (a) in a dry state, the bilayer remains unfolded; (b) in an aqueous environment the polymer rolls to form a tube.

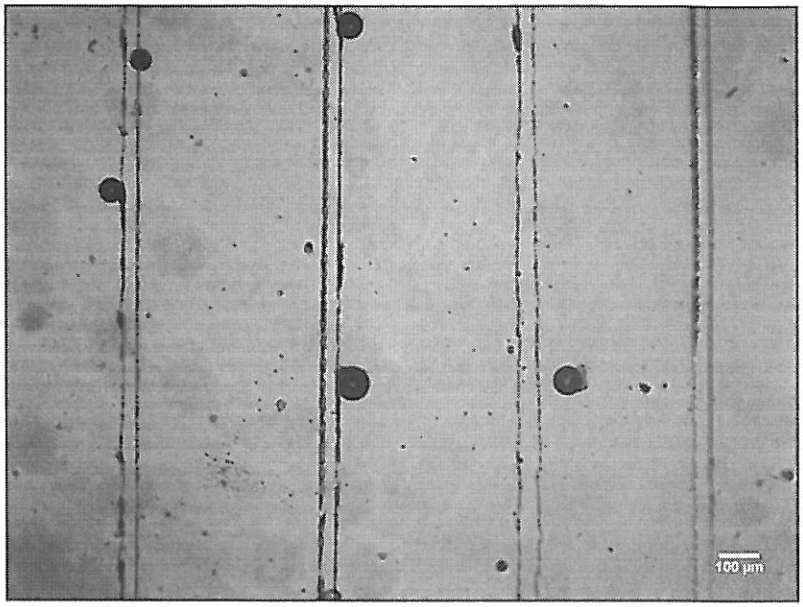

Figure 6: Patterned polymer bilayer in aqueous environment. Each section was about 300 µm wide.

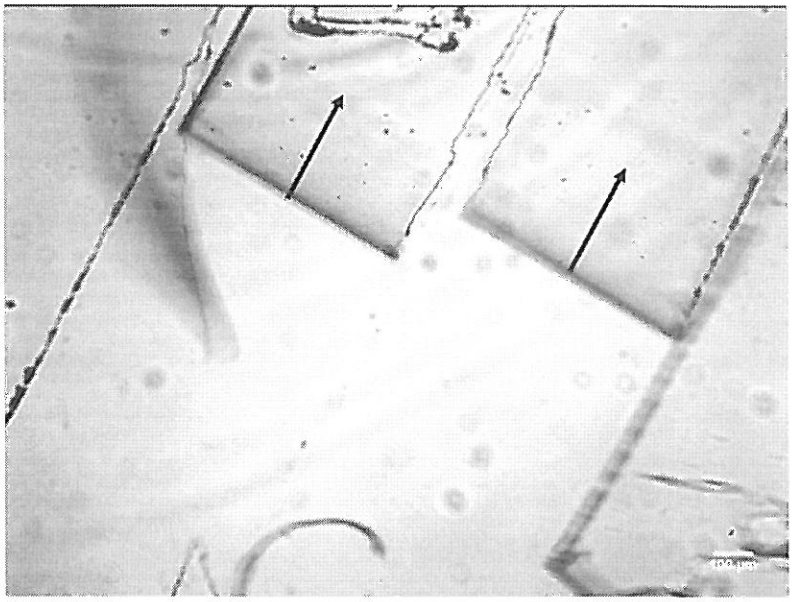

Figure 7: Temperature of aqueous environment increased (from 22°C to 32°C), tubes begun to roll.

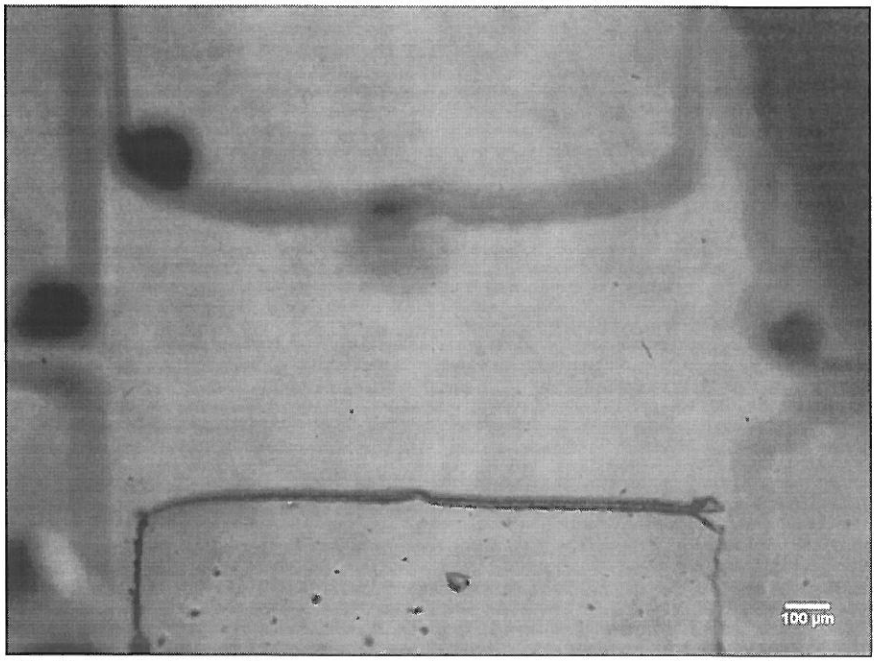

Figure 8: Temperature of aqueous environment increased to body temperature (i.e., 37 C), polystyrene layer detaches from glass substrate.

In figure 6, the polymer bilayer remains undeformed in an aqueous environment at room temperature which is contrary to the expected result of tube formation. Then, in figure 7 the temperature is slowly increased from room temperature which causes the tube to begin rolling end over end because the swelling of gelatin increases with temperature. Finally, the aqueous environment reaches body temperature in figure 8, this causes the active gelatin layer to dissolve and the polystyrene to detach from the glass substrate and become undeformed once again.

Formation of Polymer Tube by Removal of Sugar Fiber

This study also investigated the formation of polymer tubes by coating sugar fibers and then removing the fiber by placing the polymer coated fiber in an aqueous environment. This method begins by heating the pure sugar in order to melt the sugar down into a liquid, then the tip of a needle was used to draw out a sugar fiber from the liquid sugar. The initial fiber can be seen in figure 9.

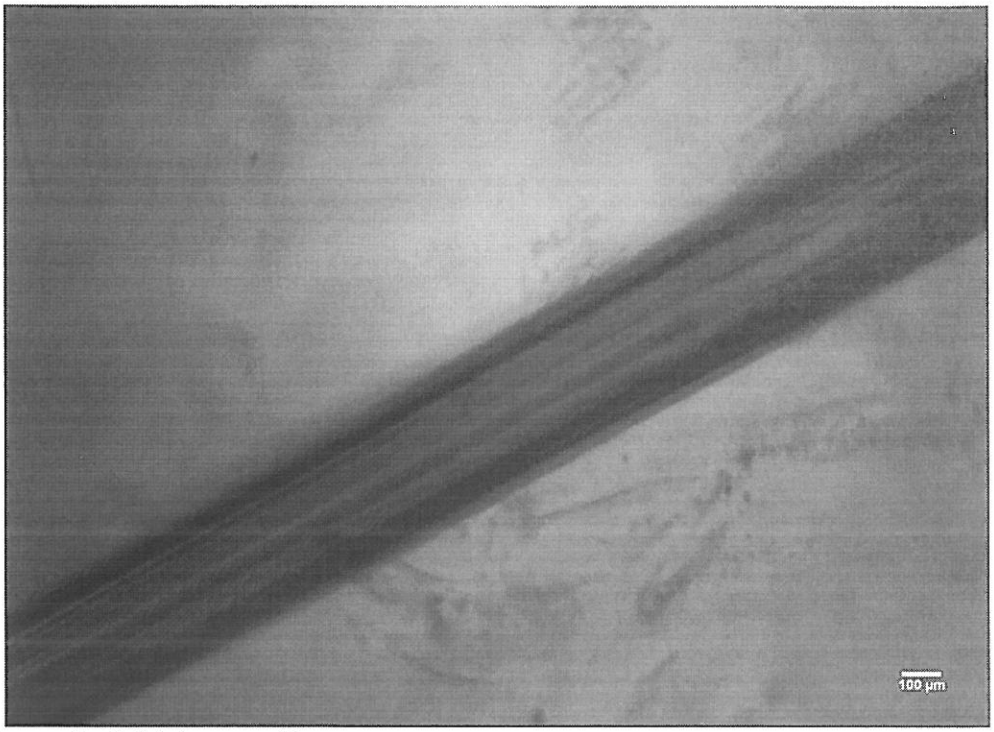

Figure 9: Initial sugar fiber.

After the initial fiber was drawn out, the fiber would then be coated with a biocompatible and biodegradable polymer known as poly (lactide-co-glycolide). This polymer was used because it had a low glass transition temperature and it has been approved for use in biotechnological applications. The polymer coated sugar fiber can be seen in figure 10.

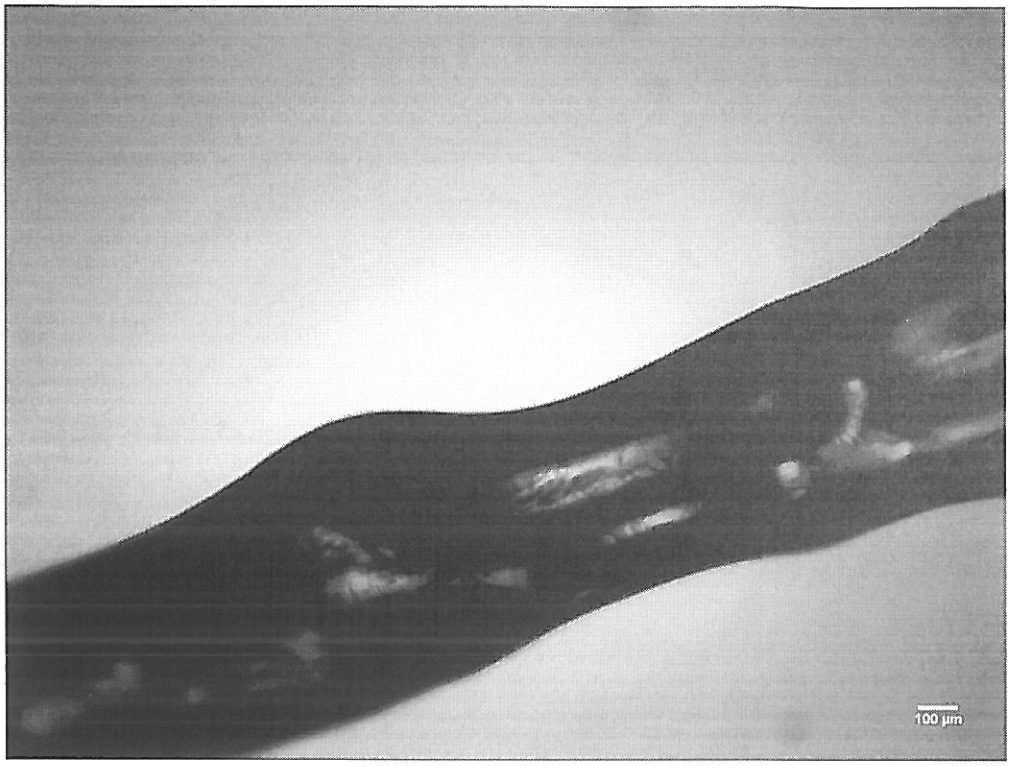

Figure 10: PLGA coated sugar fiber.

In order to form a hollow polymer tube, the polymer coated sugar fiber is introduced in to an aqueous environment. This environment causes the sugar fiber to dissolve and with its removal, a hollow polymer tube is formed. An image of the polymer tube formed by the removal of the sugar fiber can be seen in figure 11.

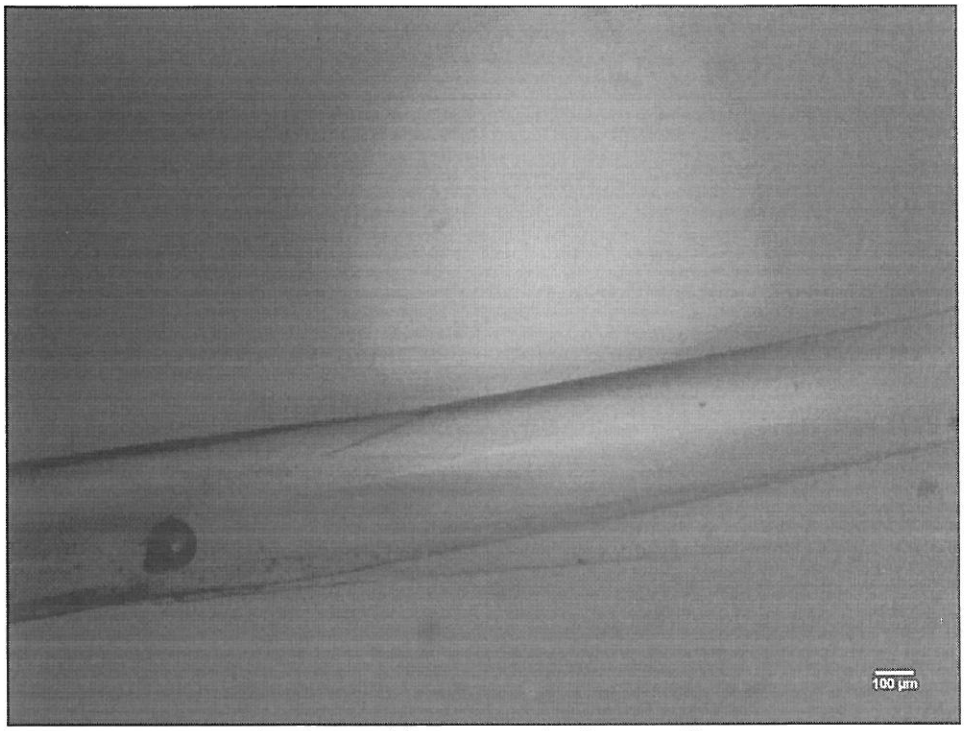

Figure 11: Polymer tube formed by removal of sugar fiber.

The tubes formed by the removal of the sugar fiber had an average inner diameter of 232.9 micrometers with a standard deviation of 23.9, which is too large for use in tissue engineering as the polymer tubes need to be on the order of microns. In order to find the diameter of each tube, ten measurements were taken of each tube and averaged. Figure 12 shows the distribution of the outer diameter measurements for the polymer tubes

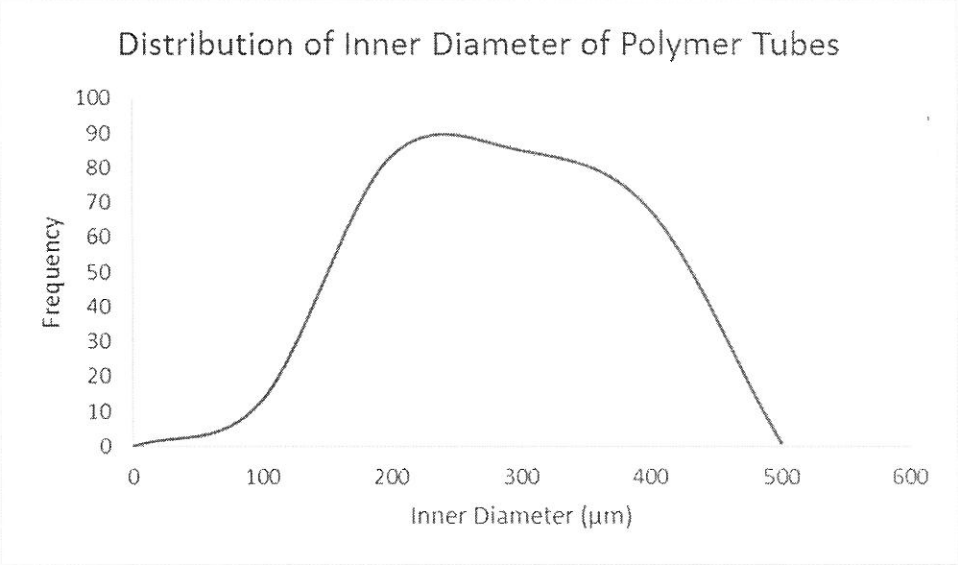

Figure 12: Distribution of inner diameter of polymer tubes.

Additionally, the tubes that were formed by the removal of sugar were further tested to see if cells could be injected through a syringe and survive within the tube. In this study we placed the needle and polymer tube underneath an ultraviolet light for fifteen minutes in order to sterilize the tube before cell injection. When the cells were injected into the polymer tube, the tube ruptured due to the pressure of injection. In figure 13, a picture of the ruptured tube is shown.

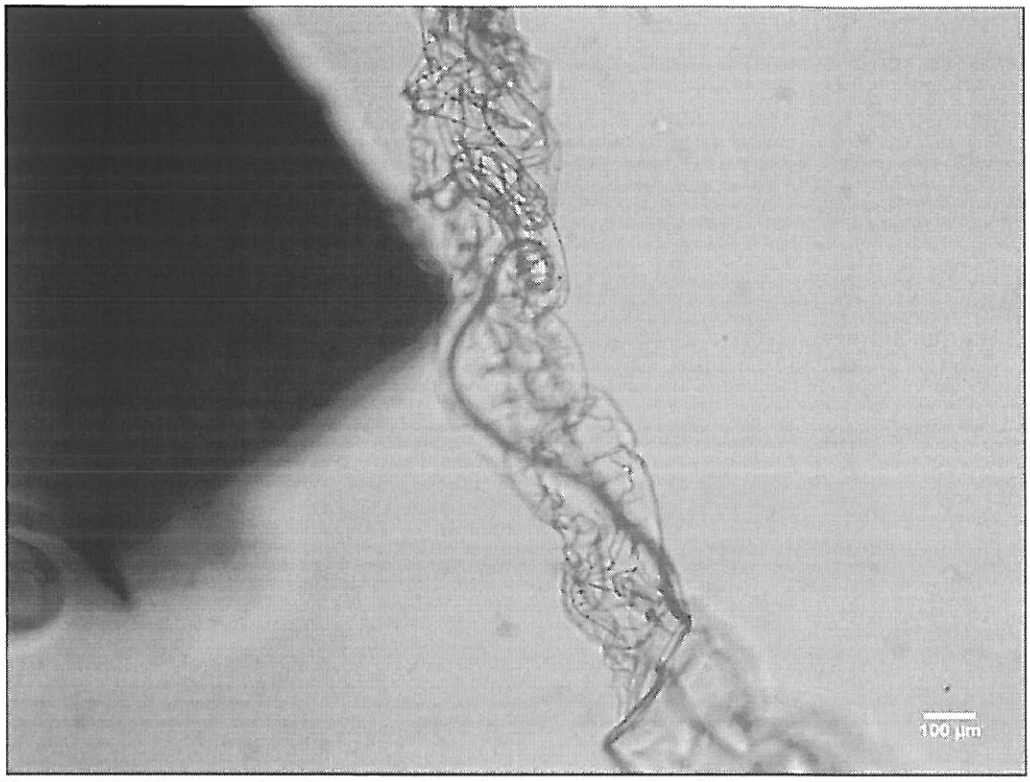

Figure 13: Ruptured polymer tube after attempted cell injection.

Discussion/Analysis

The use of thermo-triggered self-rolled polymer tubes has not been proven to work in this study. The polymer bilayer did not self-roll at room temperature as predicted but, the polymer bilayer did begin to roll once the temperature was increased from room temperature. Additionally, the active gelatin layer dissolved once the aqueous environment reached body temperature, which was the expected observation. Further research should be done using different polymers as the different chemical and mechanical properties of these polymers may lead to successful formation of self-rolled polymer micro-tubes. Also, further research needs to be done on polymer bilayers that react to different stimuli such as ultraviolet light and pH.

This study also investigated the formation of polymers through the removal of a sugar fiber by introduction to an aqueous environment. It was found that this method did form polymer tubes with diameters in the micrometer range. Therefore, further research needs to be done by decreasing the diameter of sugar fiber and attempting to insert cells within the hollow polymer tube so, it can be used for tissue engineering.

The results suggest that polymer tubes can be formed through the removal of a sugar fiber by dissolving the fiber in deionized water. This method also allows the tube to be formed on the tip of a needle, facilitating the insertion of cells into the polymer tube. Therefore, this study has found that thermos-triggered self-folding polymer tubes cannot be formed using a polymer bilayer containing gelatin and polystyrene and that polymer tubes can be formed through the removal of a sugar fiber.

Appendices

Table A.1: Scale used to measure tube diameter.

Scale		
µm	pixels	pixels/µm
1000	820	0.82

Table A.2: Inner Diameter Measurements of Polymer Tubes.

Measurement #	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	Tube 8	Tube 9	Tube 10	Tube 11	Tube 12	Tube 13
1	353.04	280.12	225.679	232.63	369.07	132.33	325.25	373.90	292.74	235.812	127.194	119.727	123.83
2	348	280.04	269.592	238.41	370.08	121.61	311.94	357.48	294.94	251.438	138.671	128.867	116.785
3	351.27	282.84	266.619	244.30	358.20	119.13	319.44	377.13	286.68	247.299	124.381	122.55	104.984
4	348.35	294.3	262.075	235.11	366.03	126.6	324.59	368.17	289.80	244.189	123.83	118.905	93.347
5	334.87	292.93	246.74	245.60	362.87	124.44	303.54	380.56	262.09	246.335	125.144	115.864	101.644
6	341.6	288.77	234.227	236.58	360.85	116.16	311.61	380.50	265.30	260.968	115.684	109.612	106.251
7	349.05	296.31	217.712	228.58	358.24	122.33	308.73	374.54	282.25	239.206	124.93	116.785	115.684
8	355.14	305.09	206.021	243.90	368.78	117.76	303.54	376.01	280.75	226.826	117.975	114.652	102.141
9	344.3	286.35	267.165	239.58	356.87	117.23	318.01	369.75	276.28	239.007	116.708	110.423	110.423
10	330.77	282.12	237.354	268.90	364.96	120.03	308.00	362.15	285.26	236.568	117.799	114.314	115.864

Table A.3: Inner Diameter Measurements of Polymer Tubes.

Measurement #	Tube 14	Tube 15	Tube 16	Tube 17	Tube 18	Tube 19	Tube 20	Tube 21	Tube 22	Tube 23	Tube 24	Tube 25
1	148.849	110.504	76.542	393.675	354.464	299.898	267.44	249.025	230.145	193.654	183.368	179.367
2	146.736	108.768	78.65	382.906	334.264	287.101	270.723	262.683	232.523	183.368	190.62	168.58
3	139.612	104.187	80.519	383.054	333.061	295.291	269.82	248.439	216.495	199.821	197.29	176.896
4	141.789	108	78.537	391.136	319.637	286.167	257.665	252.099	220.105	194.65	191.321	175.901
5	124.572	105.802	89.443	390.52	324.082	313.057	278.007	253.499	218.273	200	176.492	177.55
6	135.284	109.205	79.888	396.932	328.385	309.762	284.269	246.263	235.446	204.703	189.305	174.595
7	139.676	97.095	95.396	399.926	331.871	303.771	263.542	230.545	224.188	198.747	185.737	171.241
8	133.068	93.856	92.032	395.123	325.474	314.601	262.15	243.213	228.914	206.626	184.145	163.857
9	127.685	118.93	88.675	378.469	340.994	293.736	281.24	253.182	230.609	220.645	179.88	188.471
10	140.715	119.975	73.732	397.022	337.593	292.376	271.195	230.7	204.194	220.091	187.029	184.87
	137.7986	107.6322	83.3414	390.8763	332.9825	299.576	270.6051	246.9648	224.0892	202.2305	186.5187	176.1328

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